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Iron on the brain

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Accumulations of iron are often detected in the brains of people suffering from neurodegenerative diseases. But it is often not known whether such accumulations contribute directly to disease progression. The identification of the genes mutated in two such disorders suggests that errors in iron metabolism do indeed have a key role.

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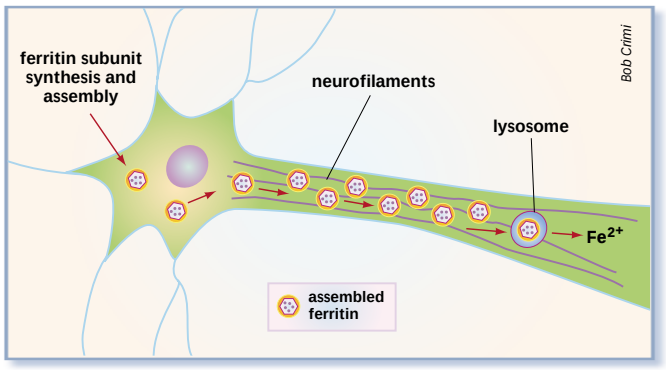
For many years it has been well accepted that accumulations of iron in organs such as the liver and heart can cause disease. In disorders such as genetic hemochromatosis and thalassemia, hepatic iron overload causes cirrhosis; cardiac iron overload leads to heart failure[1]. Accumulations of iron are also frequently observed in areas of the brain that degenerate in disorders such as Parkinson and Alzheimer diseases. But the importance of iron accumulation in the progression of most neurodegenerative diseases has been unclear[2], in part because all aging humans normally accumulate iron in brain regions such as the substantia nigra. On pages 327 and 345 of this issue, Curtis and colleagues[3] and Zhou and co-workers[4] provide some answers. They describe how they used genetics and positional cloning to identify the genes defective in two neurodegenerative diseases that are characterized by profound accumulations of iron in the brain.



Iron and neurodegeneration. A model of iron damage to axons. Ferritin is an iron-storage protein. Ferritin subunits are synthesized in the neuronal cell body, and mature, assembled heteropolymers are found within axons[6]. Some of the ferritin in distal axons and presynaptic terminals are degraded within lysosomes, potentially releasing ferrous iron into a region of the neuron in which proteins, such as components of neurofilaments, are vulnerable to iron-binding and oxidative damage. Alternatively, ferritin heteropolymers that contain abnormal subunits spontaneously release free iron into the axon or synapse.

Curtis *et al.*[3] first describe a previously unrecognized, adult-onset neurodegenerative disease that affects the basal ganglia and is associated with iron accumulation. They then identify a dominantly inherited gene that is mutated in the affected individuals, who are from northern England. All of the patients have the same mutation—insertion of an adenine within the portion of the gene that encodes the carboxy terminus of the L chain of ferritin, an iron-storage protein. The mutation leads to synthesis of a unique 22-amino-acid carboxy terminus.

In the brains of these patients, the globus pallidus shows abundant spherical inclusions that contain ferritin. Throughout the white matter of the brain, axonal swellings are immunoreactive for neurofilaments, ubiquitin and tau—a characteristic of neurodegenerative diseases. Interestingly, however, serum ferritin levels are remarkably low, and the pancreas, liver and heart appear to function normally. So, although the abnormal ferritin is almost certainly ubiquitously expressed, it would seem that only neurons develop significant pathology. The authors propose that this disorder should be referred to as 'neuroferritinopathy'.

On the basis of the crystal structure of ferritin, Curtis *et al.*[3] suggest that the altered carboxy terminus may affect the protein's function and stability. Ferritin is a heteropolymeric iron-storage protein, composed of H and L subunits that assemble to form a hollow sphere in which ferric iron precipitates are sequestered[5]. The carboxy terminus of the aberrant L chain might interfere with stable polymer formation, perhaps allowing inappropriate release of iron from iron-laden ferritin.

Interestingly, the simultaneous overexpression of the ferritin H and L chains is implicated in the progression of a newly described neurodegenerative disease in mice that lack iron-regulatory protein-2 (IRP2)[6]. Here, axonal degeneration is the earliest pathological event identified in neurons that overexpress ferritin. Immunohistochemical staining of wildtype and *Irp2*$^{-/-}$ mice revealed ferritin throughout the length of axons, hinting that ferritin is normally transported from its site of synthesis at cell bodies to synapses. In *Irp2*$^{-/-}$ mice, however, the levels of ferric iron in axons are markedly increased, and much of this iron is probably sequestered within the overexpressed ferritin.

There could be many reasons why neurons degenerate in the newly described human neuroferritinopathy and in *Irp2*$^{-/-}$ mice. But it is attractive to consider models that could be applied to both diseases. It is difficult to ascertain where the initial neuronal damage occurs in humans[3], but the studies of *Irp2*$^{-/-}$ mice[6] point to the axon. The transport of ferritin down the axon might therefore be the key to the pathology associated with the overexpression of both ferritin subunits in *Irp2*$^{-/-}$ mice, and with the production of the abnormal ferritin L chain in humans with neuroferritinopathy.

Neurons are highly polarized, with proteins being synthesized in the cell body and then transported over potentially long distances to synapses. Ferritin probably sequesters iron in the cell body, but transport of ferritin down the axon may allow net transport of iron to synapses. Normally, the half-life of ferritin appears to be determined by its degradation in lysosomes[7]; in the *Irp2*$^{-/-}$ mice, the degradation of overexpressed ferritin would lead to the increased release of free iron. This could occur within lysosomes present in distal axons[8]. The positively charged iron atoms could then bind to axonal proteins, including negatively charged components of neurofilaments, causing

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oxidation and loss of function. In people with neuroferritinopathy, perhaps the ferritin heteropolymers containing the aberrant L chain are inherently unstable; here, the release of iron might also occur spontaneously as ferritin is transported down the axon. Thus, iron-dependent oxidative damage to the axon may be an early event that is common to both diseases. The highly polarized nature of the neuron, together with axonal trafficking of iron-laden ferritin, may explain why significant pathology is seen only in the nervous system.

Meanwhile, Zhou *et al.*[4] have identified the defective gene that causes one of the neurodegenerative diseases in which iron accumulation is most dramatic — an autosomal, recessively inherited disease known until recently as Hallervorden–Spatz disease. Adolescents and young adults with this disease develop a progressive, disabling movement disorder, characterized by spasmodic and uncontrollable movements of the trunk and limbs and by distorted body positions. In autopsied brains, accumulations of iron in the globus pallidus and pars reticulata of the substantia nigra are profound.

Zhou *et al.* detected the underlying mutations in a gene that encodes pantothenate kinase. This enzyme is essential in coenzyme A biosynthesis, and catalyses the phosphorylation of pantothenate (vitamin B5) and related substrates. The product of this reaction, 4′-phosphopantothenate, is then converted to 4′-phosphopanthetheine in a reaction that consumes cysteine. Humans have four genes encoding pantothenate kinases, and the one identified here, *PANK2*, appears to be expressed specifically in the brain, potentially explaining the pattern of pathology in specific regions of the central nervous system.

Moreover, given that cysteine is consumed in the conversion of 4′-phosphopantothenate, an absence of functional *PANK2* might also explain the previously observed accumulation of cysteine in the degenerating brain areas of patients with Hallervorden–Spatz disease[9]. In addition, the well-known iron-chelating properties of cysteine might account for the observed regional iron accumulations, and cysteine-bound iron may promote iron-dependent oxidative damage in these regions. So, although pantothenate kinase-2 is not directly involved in iron metabolism, its absence may contribute to secondary iron accumulation, the consequence of which might still be important in the disease process.

The best news to come from the identification of this disease gene is that the mutations are loss-of-function mutations, so delivery of 4′–phosphopantothenate or coenzyme A might prevent neurodegeneration. The generation of *Pank2*–null mice will allow treatment strategies to be evaluated.

There is still much to learn about the basic biology of this disease, which Zhou *et al.*[4] suggest should be called 'pantothenate-kinase-associated neurodegeneration', or 'PKAN'. Likewise, the mechanisms underlying the neuroferritinopathy described by Curtis *et al.*[3] remain to be determined. However, these discoveries may lead to new insights into these neurodegenerative diseases and to the design of specific treatments—and, not least, to respect for the importance of the regulation of iron metabolism in cells that must survive and function over the lifespan of human beings. □

1. Sheth, S. & Brittenham, G. M. *Annu. Rev. Med.* **51**, 443–464 (2000).
2. Qian, Z.M. & Shen, X. *Trends Mol. Med.* **7**, 103–108 (2001).
3. Curtis, A.R. J. *et al. Nature. Genet.* **28**,327–334 (2001).
4. Zhou, B. *et al. Nature. Genet.* **28**, 345–349 (2001).
5. Harrison, P.M. & Arosio, P. *Biochim. Biophys. Acta* **1275**, 161–203 (1996).
6. LaVaute, T. *Nature Genet.* **27**, 209–214 (2001).
7. Radisky, D.C. & Kaplan, J. *Biochem. J.* **336**, 201–205 (1998).
8. Overly, C.C. & Hollenbeck, P.J. *J. Neurosci.* **16**, 6056–6064 (1996).
9. Perry, T.L. *et al. Ann. Neurol.* **18**, 482–489 (1985).

Eggs in the balance

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Polycyclic aromatic hydrocarbons (PAH), found in cigarette smoke and air pollution, interact with the aryl hydrocarbon receptor (Ahr) to cause reproductive defects. Mice lacking either Ahr or the pro-apoptotic protein Bax have an increased number of primordial follicles, and these mutant oocytes are resistant to PAH toxicity. A new study shows that the *Bax* promoter contains two core Ahr response elements, which are required for PAH stimulation of *Bax* promoter activity in oocytes. Thus, the toxic effects of PAH in oocytes are mediated directly by Ahr induction of the Bax pathway.

Twenty years ago, Mattison[1] showed that PAH xenobiotics, including 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxins (TCDD) and benzo(a)pyrene (a potent carcinogen and mutagen in cigarettes), cause oocyte loss. PAH exert their effects through the aryl hydrocarbon receptor (Ahr), a ligand-activated member of the Per-ARNT-Sim (PAS) transcription factor family that also contains a basic helix-loop-helix DNA-binding domain[2]. Upon ligand binding to its PAS domain, cytoplasmic Ahr translocates to the nucleus, where it dimerizes with the Ahr nuclear translocator and directly activates gene expression. Mice lacking Ahr are viable and can produce offspring, but have smaller livers and many hepatic pathologies[3]. In addition, they do not respond to TCDD by induction of the cytochrome P450, 1a1, and show only a 25% induction of the cytochrome P450, 1a2, compared with wildtype controls. Thus, induction of these cytochromes by TCDD requires Ahr.

Members of the Bcl2 family regulate apoptosis and are found in species as divergent as nematodes (for example, *Caenorhabditis elegans*) and humans. The Bcl2 and Bclx subgroup is anti-apoptotic, whereas the Bax subgroup is pro-apoptotic. *Bcl2*$^{-/-}$ mice have kidney, lymphocyte, and hair abnormalities, and half die by six weeks of age[4]. *Bax*$^{-/-}$ mice have B- and T-cell hyperplasia, and a block in spermatogenesis leading to male infertility[5]. On page 355 of this issue, Tiina Matikainen and colleagues[6] show a direct link between Ahr and Bax in the regulation of oocyte survival.